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<b>(54) Title:</b> SYNERGISTIC WATER SOLUBLE PRESERVATIVE COMPOSITIONS OF BIOCIDAL MIXTURES  <b>(57) Abstract</b>  A water soluble preservative admixture of biocidal compounds for addition to commercial use compositions at predetermined use levels, and uniformly distributed therein, to provide long-time synergistic biocidal activity against a wide range of fungi and both gram-negative and gram-positive bacteria, which comprises powders of (a) one or more methylol compounds, or their equivalents, and (b) iodopropynyl alcohol, or its ester, carbamate or ether derivative thereof, in a weight ratio of (a):(b) of 100:1 to 2000:1.		

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SYNERGISTIC WATER SOLUBLE PRESERVATIVE  
COMPOSITIONS OF BIOCIDAL MIXTURES

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a water soluble preservative admixture for addition to commercial use formulations to provide long time synergistic biocidal activity therein, and, more particularly, to admixtures of a methylol compound and an iodopropynyl compound, in predetermined weight ratios of 100:1 to 2000:1.

2. Description of the Prior Art

Combinations of antimicrobial agents have been developed in the prior art in order to:

- (1) produce a biochemical synergism;
- (2) broaden the antimicrobial spectrum of activity of each agent;
- (3) increase water solubility for the admixture;
- (4) minimize the toxicity or irritation of a given agent to the host; and
- (5) minimize physical and chemical incompatibilities.

True biological synergism exists when two agents, when combined, require lesser amounts of the agents to bring about the same inhibitory or cidal effect than either single agent alone. While synergistic interaction for two or more antimicrobial agents does produce more than merely an additive effect in the resultant biological activity, in most cases the mechanism of such synergism remains a mystery.

M. Rosen et al., in U.S. Patent 4,844,891, for example, described a preservative admixture of (a) a formaldehyde donor and (b) a halopropynyl compound, in a weight ratio of (a):(b) of 50:1 to 1:1, preferably 30:1 to 2:1, and, most preferably, 20:1 to 10:1, as providing fungicidal activity for 1-3 days in commercial use formulations. However, Rosen observed that when the ratio of (a):(b) in the concentrate exceeded 50:1 (System No. 16 in Table 1, a ratio of 73.33), the preservative composition was ineffective in providing biocidal protection in the use formulations. Thus a relatively large amount of the halopropynyl compound was required by Rosen to provide significant biocidal activity in the use composition. In such admixtures, although the formaldehyde donor is water soluble, the halopropynyl compound is substantially insoluble in water. Therefore it was difficult for Rosen to uniformly distribute his admixture throughout the use composition.

For these and other reasons, it is desired to provide a new and improved water soluble preservative admixture of such biocidal compounds which requires relatively little of the water insoluble and expensive halopropynyl compound, and that also provides effective synergistic protection in use formulations against a wide range of fungi and bacteria at different use levels over a long period of time.

A feature of the present invention is the provision of an admixture concentrate which is water soluble and which therefore can be uniformly distributed in use compositions at a predetermined use level.

Another feature herein is the provision of a preservative admixture which exhibits a long term synergistic biocidal activity against wide range of fungi and both gram-negative and gram-positive bacteria in the use compositions.

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Still another feature of this invention is the provision of a water soluble preservative admixture for personal care compositions in the form of a solution, lotion, gel, emulsion, emulsifiable concentrate, suspension, slurry or cream.

These and other objects and features of the invention will be made apparent from the following more particular description of the invention.

#### SUMMARY OF THE INVENTION

What has been discovered is a water soluble preservative composition for addition to commercial use compositions at predetermined use levels, and uniformly distributed therein, which provides long term synergistic biocidal activity against a wide range of fungi and both gram-negative and gram-positive bacteria. The composition of the invention comprises an admixture of powders of

- (a) a methylol compound, or their equivalent, and
  - (b) iodopropynyl alcohol, or its ester, carbamate or ether derivative thereof,
- in a weight ratio of (a):(b) of 100:1 to 2000:1, preferably 200:1 to 500:1.

Commercial use compositions containing about 0.01 to 2% by weight of the composition of the invention also are provided therein. Such use compositions contain an iodopropynyl compound in an amount of about 0.5 to 10 ppm, to provide the desired antifungal activity, and a methylol compound, or equivalent thereof, in an amount of at least 250 ppm, to provide the desired antibacterial activity.

In another embodiment of the invention the composition also includes propylene glycol or 1,3-butylene glycol.

### DETAILED DESCRIPTION OF THE INVENTION

The invention is based upon the discoveries that in a predetermined admixture of (a) a methylol compound, or its equivalent, and (b) an iodopropynyl compound:

(1) Iodopropynyl compounds are substantially water insoluble at weight ratios of (a):(b) of less than 100; accordingly, at weight ratios below 100:1, it is difficult to uniformly distribute the iodopropynyl compound in aqueous use compositions, particularly in creams, gels and the like. In this invention, the admixtures are used at a weight ratio of (a):(b) of 100:1 to 2000:1, which are water soluble in all use compositions at conventional use levels.

(2) Effective synergistic biocidal activity is achieved for admixtures having a weight ratio of (a):(b) of 100:1 to 2000:1. Such admixtures have a Synergistic Index (SI) value approaching zero (maximum synergism) for a wide range of organisms. In contrast, admixtures with (a):(b) ratios below 100:1, e.g. 10:1 to 50:1, are much less synergistic, and are active with only a narrower range of organisms.

(3) Preservative activity for use compositions is achieved most effectively with an admixture wt. ratio of 100:1 to 2000:1 at use levels of 0.05 to 2% by weight of the finished product. In this amount, the iodopropynyl compound is present in an amount of only 0.5-10 ppm, which significantly reduces the cost and toxicity of the use composition. The methylol compound also is present in an amount of at least 250 ppm.

The experimental results upon which these discoveries are based are described below. In these examples, the (a) methylol compound may be selected from diazolidinyl urea (GERMALL® II) N-[1,3-bis(hydroxymethyl)-2,5-dioxo-4-imidazolidinyl]-N,N'-bis(hydroxymethyl) urea, imidurea (GERMALL 115),

1,3-dimethylol-5,5-dimethyl hydantoin (DMDMH), sodium hydroxymethylglycinate (SUTTOCID A), glycine anhydride dimethylol (GADM), dimethylhydroxymethyl pyrazole, (1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride (a methylol equivalent), 1,3,5-(trishydroxyethyl)hexahydrotriazine, or hydroxymethyl pyrrolidone; and the (b) iodopropynyl compound is iodopropynyl alcohol (IPGA) or 3-iodo-2-propynylbutyl carbamate (IPBC).

#### 1. WATER SOLUBILITY

The water solubility or insolubility of admixtures of several methylol compounds with IPBC as 1% aqueous solutions is shown in Tables A through C below.

TABLE A

Weight Ratio of Germall® II:IPBC	Amount in ppm	Solubility
2000:1	5	Soluble
1000:1	10	Soluble
500:1	20	Soluble
200:1	50	Soluble
100:1	100	Soluble
50:1	200	Insoluble
20:1	500	Insoluble

TABLE B

Weight Ratio of GADM:IPBC	Amount in ppm	Solubility
2000:1	5	Soluble
1000:1	10	Soluble
500:1	20	Soluble
200:1	50	Soluble
100:1	100	Soluble
50:1	200	Insoluble
20:1	500	Insoluble

TABLE C

Weight Ratio of DMDMH:IPBC	Amount in ppm	Solubility
2000:1	5	Soluble
1000:1	10	Soluble
500:1	20	Soluble
200:1	50	Soluble
100:1	100	Soluble
50:1	200	Insoluble
20:1	500	Insoluble

These results demonstrate that admixtures having a ratio of 50:1 or 20:1 are insoluble in water whereas at ratios of 100:1 to 2000:1 the admixtures are soluble in water.

## 2. SYNERGISM

Tables 1 through 14 below demonstrate the very effective synergistic interaction between compounds "a" and "b" against a broad range of fungi and both gram-negative and gram-positive bacteria. The following organisms were tested:

Organism	ATCC	
	Number	Inoculum Concentration
Ps. aeruginosa (PSA)*	9027	2.1 x 10 <sup>6</sup> CFU/gm of Product
E. coli (ECOLI)*	8739	4.7 x 10 <sup>5</sup> CFU/gm of Product
Staph. aureus (SA)**	6538	1.6 x 10 <sup>6</sup> CFU/gm of Product
Ps. cepacia (PC)*	25416	1.6 x 10 <sup>6</sup> CFU/gm of Product
C. albicans (CAN)***	10231	8.0 x 10 <sup>4</sup> CFU/gm of Product
A. niger (AN)***	16404	2.7 x 10 <sup>5</sup> CFU/gm of Product

Table D below lists the static (MIC) and cidal activities of several antimicrobial agents in ppm. These activities are used to calculate the Synergism Index (SI) of admixtures of the present invention.

- 
- \* gram-negative bacteria  
 \*\* gram-positive bacteria  
 \*\*\* fungi



TABLE D

Static (MIC) and Cidal Activities of Several Antimicrobial Compounds  
(Static/Cidal Concentrations in ppm)

Organism (ATCC #)	IPBC	Germallol II	GADM	DMDMH	IPGA
(SA) (6538)	100/200	400/1600	400/800	450/1600	300/5000
(ECOLI) (8739)	50/100	400/1600	400/800	400/800	150/600
(PSA) (9027)	800/1200	400/1600	400/400	600/1600	70/70
(PC) (25416)	1200/1800	200/400	200/400	600/1600	70/300
(CAN) (10231)	50/100	1500/15000	7500/15000	8000/16000	50/300
(AN) (16404)	50/100	3200/3200	1600/3200	8000/16000	30/30

The Synergism Index was determined by the same mathematical treatment of such data described by Kull et al. in Applied Microbiology 9,538-541 (1961) using the following relationship:

$$\text{Synergism Index (SI)} = \frac{Q_A}{Q_a} + \frac{Q_B}{Q_b}$$

where:

1.  $Q_a$  = The quantity of Compound a acting alone, producing an endpoint.
2.  $Q_b$  = The quantity of Compound b acting alone, producing an endpoint.
3.  $Q_A$  = The quantity of Compound A in mixture, producing an endpoint.
4.  $Q_B$  = The quantity of Compound B in mixture, producing an endpoint.

When SI is equal to 1, a mere additive effect of the components in the mixture is indicated; when SI is less than 1, synergism has occurred; and when SI is greater than 1 it indicates antagonism of the two components.

According to this well known method of measuring synergism, the quantity of each component in the various mixtures is compared with the quantity of pure component that is required to reach the same endpoint or to produce the same microbiological effect as the mixture.

TABLE 1  
2000:1 Wt. Ratio GERMALL® II/IPBC

Use Level	Organism	$Q_a$	$Q_b$	$Q_A$	$Q_B$	SI
0.01%	SA	200	1600	0.05	99.95	0.06
"	ECOLI	100	1600	0.05	99.95	0.06
"	PSA	1200	1600	0.05	99.95	0.06
"	PC	1800	1250	0.05	99.95	0.08
"	CAN	100	15000	0.05	99.95	0.01
"	AN	100	3200	0.05	99.95	0.03

Use Level	Organism	$Q_a$	$Q_b$	$Q_A$	$Q_B$	SI
0.025%	SA	200	1600	0.125	249.9	0.16
"	ECOLI	100	1600	0.125	249.9	0.16
"	PSA	1200	1600	0.125	249.9	0.16
"	PC	1800	1250	0.125	249.9	0.20
"	CAN	100	15000	0.125	249.9	0.02
"	AN	100	3200	0.125	249.9	0.08

Use Level	Organism	$Q_a$	$Q_b$	$Q_A$	$Q_B$	SI
0.05%	SA	200	1600	0.25	499.8	0.31
"	ECOLI	100	1600	0.25	499.8	0.31
"	PSA	1200	1600	0.25	499.8	0.31
"	PC	1800	1250	0.25	499.8	0.40
"	CAN	100	15000	0.25	499.8	0.04
"	AN	100	3200	0.25	499.8	0.16

Use Level	Organism	$Q_a$	$Q_b$	$Q_A$	$Q_B$	SI
0.10%	SA	200	1600	0.5	999.5	0.63
"	ECOLI	100	1600	0.5	999.5	0.63
"	PSA	1200	1600	0.5	999.5	0.63
"	PC	1800	1250	0.5	999.5	0.80
"	CAN	100	15000	0.5	999.5	0.07
"	AN	100	3200	0.5	999.5	0.32

TABLE 1 (CONT)

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.20%	SA	200	1600	1	1999	1.25
"	ECOLI	100	1600	1	1999	1.26
"	PSA	1200	1600	1	1999	1.25
"	PC	1800	1250	1	1999	1.60
"	CAN	100	15000	1	1999	0.14
"	AN	100	3200	1	1999	0.63

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.40%	SA	200	1600	2	3998	2.51
"	ECOLI	100	1600	2	3998	2.52
"	PSA	1200	1600	2	3998	2.50
"	PC	1800	1250	2	3998	3.20
"	CAN	100	15000	2	3998	0.29
"	AN	100	3200	2	3998	1.27

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.50%	SA	200	1600	2.5	4997.5	3.14
"	ECOLI	100	1600	2.5	4997.5	3.15
"	PSA	1200	1600	2.5	4997.5	3.13
"	PC	1800	1250	2.5	4997.5	4.00
"	CAN	100	15000	2.5	4997.5	0.36
"	AN	100	3200	2.5	4997.5	1.59

TABLE 2  
1000:1 Wt. Ratio GERMALL® II/IPBC

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.01%	SA	200	1600	0.1	99.9	0.06
"	ECOLI	100	1600	0.1	99.9	0.06
"	PSA	1200	1600	0.1	99.9	0.06
"	PC	1800	1250	0.1	99.9	0.08
"	CAN	100	15000	0.1	99.9	0.01
"	AN	100	3200	0.1	99.9	0.03

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.025%	SA	200	1600	0.25	249.8	0.16
"	ECOLI	100	1600	0.25	249.8	0.16
"	PSA	1200	1600	0.25	249.8	0.16
"	PC	1800	1250	0.25	249.8	0.20
"	CAN	100	15000	0.25	249.8	0.02
"	AN	100	3200	0.25	249.8	0.08

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.05%	SA	200	1600	0.5	499.5	0.31
"	ECOLI	100	1600	0.5	499.5	0.32
"	PSA	1200	1600	0.5	499.5	0.31
"	PC	1800	1250	0.5	499.5	0.40
"	CAN	100	15000	0.5	499.5	0.04
"	AN	100	3200	0.5	499.5	0.16

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.10%	SA	200	1600	1	999	0.63
"	ECOLI	100	1600	1	999	0.63
"	PSA	1200	1600	1	999	0.63
"	PC	1800	1250	1	999	0.80
"	CAN	100	15000	1	999	0.08
"	AN	100	3200	1	999	0.32

TABLE 2 (CONT)

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.20%	SA	200	1600	2	1998	1.26
"	ECOLI	100	1600	2	1998	1.27
"	PSA	1200	1600	2	1998	1.25
"	PC	1800	1250	2	1998	1.60
"	CAN	100	15000	2	1998	0.15
"	AN	100	3200	2	1998	0.64

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.40%	SA	200	1600	4	3996	2.52
"	ECOLI	100	1600	4	3996	2.54
"	PSA	1200	1600	4	3996	2.50
"	PC	1800	1250	4	3996	3.20
"	CAN	100	15000	4	3996	0.31
"	AN	100	3200	4	3996	1.29

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.50%	SA	200	1600	5	4995	3.15
"	ECOLI	100	1600	5	4995	3.17
"	PSA	1200	1600	5	4995	3.13
"	PC	1800	1250	5	4995	4.00
"	CAN	100	15000	5	4995	0.38
"	AN	100	3200	5	4995	1.61

TABLE 10  
500:1 Wt. Ratio GERMALL II/IPBC

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.01%	SA	200	1600	0.2	99.8	0.06
"	ECOLI	100	1600	0.2	99.8	0.06
"	PSA	1200	1600	0.2	99.8	0.06
"	PC	1800	1250	0.2	99.8	0.08
"	CAN	100	15000	0.2	99.8	0.01
"	AN	100	3200	0.2	99.8	0.03

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.025%	SA	200	1600	0.5	249.5	0.16
"	ECOLI	100	1600	0.5	249.5	0.16
"	PSA	1200	1600	0.5	249.5	0.16
"	PC	1800	1250	0.5	249.5	0.20
"	CAN	100	15000	0.5	249.5	0.02
"	AN	100	3200	0.5	249.5	0.08

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.05%	SA	200	1600	1	499	0.32
"	ECOLI	100	1600	1	499	0.32
"	PSA	1200	1600	1	499	0.31
"	PC	1800	1250	1	499	0.40
"	CAN	100	15000	1	499	0.04
"	AN	100	3200	1	499	0.17

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.10%	SA	200	1600	2	998	0.63
"	ECOLI	100	1600	2	998	0.64
"	PSA	1200	1600	2	998	0.63
"	PC	1800	1250	2	998	0.80
"	CAN	100	15000	2	998	0.09
"	AN	100	3200	2	998	0.33

TABLE 3 (CONT)

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.20%	SA	200	1600	4	1996	1.27
"	ECOLI	100	1600	4	1996	1.29
"	PSA	1200	1600	4	1996	1.25
"	PC	1800	1250	4	1996	1.60
"	CAN	100	15000	4	1996	0.17
"	AN	100	3200	4	1996	0.66

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.40%	SA	200	1600	8	3992	2.54
"	ECOLI	100	1600	8	3992	2.58
"	PSA	1200	1600	8	3992	2.50
"	PC	1800	1250	8	3992	3.20
"	CAN	100	15000	8	3992	0.35
"	AN	100	3200	8	3992	1.33

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.50%	SA	200	1600	10	4990	3.17
"	ECOLI	100	1600	10	4990	3.22
"	PSA	1200	1600	10	4990	3.13
"	PC	1800	1250	10	4990	4.00
"	CAN	100	15000	10	4990	0.43
"	AN	100	3200	10	4990	1.66



TABLE 4  
200:1 Wt. Ratio GERMALL® II/IPBC

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.01%	SA	200	1600	0.5	99.5	0.06
"	ECOLI	100	1600	0.5	99.5	0.07
"	PSA	1200	1600	0.5	99.5	0.06
"	PC	1800	1250	0.5	99.5	0.08
"	CAN	100	15000	0.5	99.5	0.01
"	AN	100	3200	0.5	99.5	0.04

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.025%	SA	200	1600	1.25	248.75	0.16
"	ECOLI	100	1600	1.25	248.75	0.17
"	PSA	1200	1600	1.25	248.75	0.16
"	PC	1800	1250	1.25	248.75	0.20
"	CAN	100	15000	1.25	248.75	0.03
"	AN	100	3200	1.25	248.75	0.09

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.05%	SA	200	1600	2.5	497.5	0.32
"	ECOLI	100	1600	2.5	497.5	0.34
"	PSA	1200	1600	2.5	497.5	0.31
"	PC	1800	1250	2.5	497.5	0.40
"	CAN	100	15000	2.5	497.5	0.06
"	AN	100	3200	2.5	497.5	0.18

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.10%	SA	200	1600	5	995	0.65
"	ECOLI	100	1600	5	995	0.67
"	PSA	1200	1600	5	995	0.63
"	PC	1800	1250	5	995	0.80
"	CAN	100	15000	5	995	0.12
"	AN	100	3200	5	995	0.36

TABLE 4 (CONT)

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.20%	SA	200	1600	10	1990	1.29
"	ECOLI	100	1600	10	1990	1.34
"	PSA	1200	1600	10	1990	1.25
"	PC	1800	1250	10	1990	1.60
"	CAN	100	15000	10	1990	0.23
"	AN	100	3200	10	1990	0.72

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.40%	SA	200	1600	20	3980	2.59
"	ECOLI	100	1600	20	3980	2.69
"	PSA	1200	1600	20	3980	2.50
"	PC	1800	1250	20	3980	3.20
"	CAN	100	15000	20	3980	0.47
"	AN	100	3200	20	3980	1.44

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.50%	SA	200	1600	25	4975	3.23
"	ECOLI	100	1600	25	4975	3.36
"	PSA	1200	1600	25	4975	3.13
"	PC	1800	1250	25	4975	3.99
"	CAN	100	15000	25	4975	0.58
"	AN	100	3200	25	4975	1.80

TABLE 5  
100:1 Wt. Ratio GERMALL® II/IPBC

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.01%	SA	200	1600	1	99	0.07
"	ECOLI	100	1600	1	99	0.07
"	PSA	1200	1600	1	99	0.06
"	PC	1800	1250	1	99	0.08
"	CAN	100	15000	1	99	0.02
"	AN	100	3200	1	99	0.04

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.025%	SA	200	1600	2.5	248	0.17
"	ECOLI	100	1600	2.5	248	0.18
"	PSA	1200	1600	2.5	248	0.16
"	PC	1800	1250	2.5	248	0.20
"	CAN	100	15000	2.5	248	0.04
"	AN	100	3200	2.5	248	0.10

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.05%	SA	200	1600	5	495	0.33
"	ECOLI	100	1600	5	495	0.36
"	PSA	1200	1600	5	495	0.31
"	PC	1800	1250	5	495	0.40
"	CAN	100	15000	5	495	0.08
"	AN	100	3200	5	495	0.20

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.10%	SA	200	1600	10	990	0.67
"	ECOLI	100	1600	10	990	0.72
"	PSA	1200	1600	10	990	0.63
"	PC	1800	1250	10	990	0.80
"	CAN	100	15000	10	990	0.17
"	AN	100	3200	10	990	0.41

TABLE 5 (CONT)

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.20%	SA	200	1600	20	1980	1.34
"	ECOLI	100	1600	20	1980	1.44
"	PSA	1200	1600	20	1980	1.25
"	PC	1800	1250	20	1980	1.60
"	CAN	100	15000	20	1980	0.33
"	AN	100	3200	20	1980	0.82

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.40%	SA	200	1600	40	3960	2.68
"	ECOLI	100	1600	40	3960	2.88
"	PSA	1200	1600	40	3960	2.51
"	PC	1800	1250	40	3960	3.19
"	CAN	100	15000	40	3960	0.66
"	AN	100	3200	40	3960	1.64

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.50%	SA	200	1600	50	4950	3.34
"	ECOLI	100	1600	50	4950	3.59
"	PSA	1200	1600	50	4950	3.14
"	PC	1800	1250	50	4950	3.99
"	CAN	100	15000	50	4950	0.83
"	AN	100	3200	50	4950	2.05

TABLE 6  
50:1 Wt. Ratio GERMALL® II/IPBC

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.01%	SA	200	1600	2	98	0.07
"	ECOLI	100	1600	2	98	0.08
"	PSA	1200	1600	2	98	0.06
"	PC	1800	1250	2	98	0.08
"	CAN	100	15000	2	98	0.03
"	AN	100	3200	2	98	0.05

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.025%	SA	200	1600	5	245	0.18
"	ECOLI	100	1600	5	245	0.20
"	PSA	1200	1600	5	245	0.16
"	PC	1800	1250	5	245	0.20
"	CAN	100	15000	5	245	0.07
"	AN	100	3200	5	245	0.13

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.05%	SA	200	1600	10	490	0.36
"	ECOLI	100	1600	10	490	0.41
"	PSA	1200	1600	10	490	0.31
"	PC	1800	1250	10	490	0.40
"	CAN	100	15000	10	490	0.13
"	AN	100	3200	10	490	0.25

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.10%	SA	200	1600	20	980	0.71
"	ECOLI	100	1600	20	980	0.81
"	PSA	1200	1600	20	980	0.63
"	PC	1800	1250	20	980	0.80
"	CAN	100	15000	20	980	0.27
"	AN	100	3200	20	980	0.51

TABLE 6 (CONT)

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.20%	SA	200	1600	40	1960	1.43
"	ECOLI	100	1600	40	1960	1.63
"	PSA	1200	1600	40	1960	1.26
"	PC	1800	1250	40	1960	1.59
"	CAN	100	15000	40	1960	0.53
"	AN	100	3200	40	1960	1.01

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.40%	SA	200	1600	80	3920	2.85
"	ECOLI	100	1600	80	3920	3.25
"	PSA	1200	1600	80	3920	2.52
"	PC	1800	1250	80	3920	3.18
"	CAN	100	15000	80	3920	1.06
"	AN	100	3200	80	3920	2.03

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.50%	SA	200	1600	100	4900	3.56
"	ECOLI	100	1600	100	4900	4.06
"	PSA	1200	1600	100	4900	3.15
"	PC	1800	1250	100	4990	3.98
"	CAN	100	15000	100	4900	1.33
"	AN	100	3200	100	4900	2.53

TABLE 7  
20:1 Wt. Ratio GERMALL® II/IPBC

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.01%	SA	200	1600	5	95	0.08
"	ECOLI	100	1600	5	95	0.11
"	PSA	1200	1600	5	95	0.06
"	PC	1800	1250	5	95	0.08
"	CAN	100	15000	5	95	0.06
"	AN	100	3200	5	95	0.08

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.025%	SA	200	1600	12.5	237.5	0.21
"	ECOLI	100	1600	12.5	237.5	0.27
"	PSA	1200	1600	12.5	237.5	0.16
"	PC	1800	1250	12.5	237.5	0.20
"	CAN	100	15000	12.5	237.5	0.14
"	AN	100	3200	12.5	237.5	0.20

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.05%	SA	200	1600	25	475	0.42
"	ECOLI	100	1600	25	475	0.55
"	PSA	1200	1600	25	475	0.32
"	PC	1800	1250	25	475	0.39
"	CAN	100	15000	25	475	0.28
"	AN	100	3200	25	475	0.40

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.10%	SA	200	1600	50	950	0.84
"	ECOLI	100	1600	50	950	1.09
"	PSA	1200	1600	50	950	0.64
"	PC	1800	1250	50	950	0.79
"	CAN	100	15000	50	950	0.56
"	AN	100	3200	50	950	0.80

TABLE 7 (CONT)

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.20%	SA	200	1600	100	1900	1.69
"	ECOLI	100	1600	100	1900	2.19
"	PSA	1200	1600	100	1900	1.27
"	PC	1800	1250	100	1900	1.58
"	CAN	100	15000	100	1900	1.13
"	AN	100	3200	100	1900	1.59

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.40%	SA	200	1600	200	4800	4.00
"	ECOLI	100	1600	200	4800	5.00
"	PSA	1200	1600	200	4800	3.17
"	PC	1800	1250	200	4800	3.95
"	CAN	100	15000	200	4800	2.32
"	AN	100	3200	200	4800	3.50

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.50%	SA	200	1600	250	4750	4.22
"	ECOLI	100	1600	250	4750	5.47
"	PSA	1200	1600	250	4750	3.18
"	PC	1800	1250	250	4750	3.94
"	CAN	100	15000	250	4750	2.82
"	AN	100	3200	250	4750	3.98



TABLE 8  
2000:1 Wt. Ratio DMDMH/IPBC

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.01%	SA	200	1600	0.05	99.95	0.06
"	ECOLI	100	800	0.05	99.95	0.13
"	PSA	1200	1600	0.05	99.95	0.06
"	PC	1800	1600	0.05	99.95	0.06
"	CAN	100	16000	0.05	99.95	0.01
"	AN	100	16000	0.05	99.95	0.01

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.025%	SA	200	1600	0.125	249.88	0.16
"	ECOLI	100	800	0.125	249.88	0.31
"	PSA	1200	1600	0.125	249.88	0.16
"	PC	1800	1600	0.125	249.88	0.16
"	CAN	100	16000	0.125	249.88	0.02
"	AN	100	16000	0.125	249.88	0.02

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.05%	SA	200	1600	0.25	499.75	0.31
"	ECOLI	100	800	0.25	499.75	0.63
"	PSA	1200	1600	0.25	499.75	0.31
"	PC	1800	1600	0.25	499.75	0.31
"	CAN	100	16000	0.25	499.75	0.03
"	AN	100	16000	0.25	499.75	0.03

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.10%	SA	200	1600	0.5	999.5	0.63
"	ECOLI	100	800	0.5	999.5	1.25
"	PSA	1200	1600	0.5	999.5	0.63
"	PC	1800	1600	0.5	999.5	0.62
"	CAN	100	16000	0.5	999.5	0.07
"	AN	100	16000	0.5	999.5	0.07

TABLE 8 (CONT)

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.20%	SA	200	1600	1	1999	1.25
"	ECOLI	100	800	1	1999	2.51
"	PSA	1200	1600	1	1999	1.25
"	PC	1800	1600	1	1999	1.25
"	CAN	100	16000	1	1999	0.13
"	AN	100	16000	1	1999	0.13

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.40%	SA	200	1600	2	3998	2.51
"	ECOLI	100	800	2	3998	5.02
"	PSA	1200	1600	2	3998	2.50
"	PC	1800	1600	2	3998	2.50
"	CAN	100	16000	2	3998	0.27
"	AN	100	16000	2	3998	0.27

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.50%	SA	200	1600	2.5	4997.5	3.14
"	ECOLI	100	800	2.5	4997.5	6.27
"	PSA	1200	1600	2.5	4997.5	3.13
"	PC	1800	1600	2.5	4997.5	3.12
"	CAN	100	16000	2.5	4997.5	0.34
"	AN	100	16000	2.5	4997.5	0.34

TABLE 9  
1000:1 Wt. Ratio DMDMH/IPBC

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.01%	SA	200	1600	0.1	99.9	0.06
"	ECOLI	100	800	0.1	99.9	0.13
"	PSA	1200	1600	0.1	99.9	0.06
"	PC	1800	1600	0.1	99.9	0.06
"	CAN	100	16000	0.1	99.9	0.01
"	AN	100	16000	0.1	99.9	0.01

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.025%	SA	200	1600	0.25	249.8	0.16
"	ECOLI	100	800	0.25	249.8	0.31
"	PSA	1200	1600	0.25	249.8	0.16
"	PC	1800	1600	0.25	249.8	0.16
"	CAN	100	16000	0.25	249.8	0.02
"	AN	100	16000	0.25	249.8	0.02

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.05%	SA	200	1600	0.5	499.5	0.31
"	ECOLI	100	800	0.5	499.5	0.63
"	PSA	1200	1600	0.5	499.5	0.31
"	PC	1800	1600	0.5	499.5	0.31
"	CAN	100	16000	0.5	499.5	0.04
"	AN	100	16000	0.5	499.5	0.04

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.10%	SA	200	1600	1	999	0.63
"	ECOLI	100	800	1	999	1.26
"	PSA	1200	1600	1	999	0.63
"	PC	1800	1600	1	999	0.62
"	CAN	100	16000	1	999	0.07
"	AN	100	16000	1	999	0.07

TABLE 10  
500:1 Wt. Ratio DMDMH/IPBC

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.01%	SA	200	1600	0.2	99.8	0.06
"	ECOLI	100	800	0.2	99.8	0.13
"	PSA	1200	1600	0.2	99.8	0.06
"	PC	1800	1600	0.2	99.8	0.06
"	CAN	100	16000	0.2	99.8	0.01
"	AN	100	16000	0.2	99.8	0.01

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.25%	SA	200	1600	0.5	249.5	0.16
"	ECOLI	100	800	0.5	249.5	0.32
"	PSA	1200	1600	0.5	249.5	0.16
"	PC	1800	1600	0.5	249.5	0.16
"	CAN	100	16000	0.5	249.5	0.02
"	AN	100	16000	0.5	249.5	0.02

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.05%	SA	200	1600	1	499	0.32
"	ECOLI	100	800	1	499	0.63
"	PSA	1200	1600	1	499	0.31
"	PC	1800	1600	1	499	0.31
"	CAN	100	16000	1	499	0.04
"	AN	100	16000	1	499	0.04

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.10%	SA	200	1600	2	998	0.63
"	ECOLI	100	800	2	998	1.27
"	PSA	1200	1600	2	998	0.63
"	PC	1800	1600	2	998	0.62
"	CAN	100	16000	2	998	0.08
"	AN	100	16000	2	998	0.08

TABLE 10 (CONT)

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.20%	SA	200	1600	4	1996	1.27
"	ECOLI	100	800	4	1996	2.54
"	PSA	1200	1600	4	1996	1.25
"	PC	1800	1600	4	1996	1.25
"	CAN	100	16000	4	1996	0.16
"	AN	100	16000	4	1996	0.16

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.40%	SA	200	1600	8	3992	2.54
"	ECOLI	100	800	8	3992	5.07
"	PSA	1200	1600	8	3992	2.50
"	PC	1800	1600	8	3992	2.50
"	CAN	100	16000	8	3992	0.33
"	AN	100	16000	8	3992	0.33

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.50%	SA	200	1600	10	4900	3.11
"	ECOLI	100	800	10	4900	6.23
"	PSA	1200	1600	10	4900	3.07
"	PC	1800	1600	10	4900	3.07
"	CAN	100	16000	10	4900	0.41
"	AN	100	16000	10	4900	0.41

TABLE 11  
200:1 Wt. Ratio DMDMH/IPBC

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.01%	SA	200	1600	0.5	99.5	0.06
"	ECOLI	100	800	0.5	99.5	0.13
"	PSA	1200	1600	0.5	99.5	0.06
"	PC	1800	1600	0.5	99.5	0.06
"	CAN	100	16000	0.5	99.5	0.01
"	AN	100	16000	0.5	99.5	0.01

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.025%	SA	200	1600	1.25	248.75	0.16
"	ECOLI	100	800	1.25	248.75	0.32
"	PSA	1200	1600	1.25	248.75	0.16
"	PC	1800	1600	1.25	248.75	0.16
"	CAN	100	16000	1.25	248.75	0.03
"	AN	100	16000	1.25	248.75	0.03

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.05%	SA	200	1600	2.5	497.5	0.32
"	ECOLI	100	800	2.5	497.5	0.65
"	PSA	1200	1600	2.5	497.5	0.31
"	PC	1800	1600	2.5	497.5	0.31
"	CAN	100	16000	2.5	497.5	0.06
"	AN	100	16000	2.5	497.5	0.06

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.10%	SA	200	1600	5	995	0.65
"	ECOLI	100	800	5	995	1.29
"	PSA	1200	1600	5	995	0.63
"	PC	1800	1600	5	995	0.62
"	CAN	100	16000	5	995	0.11
"	AN	100	16000	5	995	0.11

TABLE 11 (CONT)

Use Level	Organism	$Q_a$	$Q_b$	$Q_A$	$Q_B$	SI
0.20%	SA	200	1600	10	1990	1.29
"	ECOLI	100	800	10	1990	2.59
"	PSA	1200	1600	10	1990	1.25
"	PC	1800	1600	10	1990	1.25
"	CAN	100	16000	10	1990	0.22
"	AN	100	16000	10	1990	0.22

Use Level	Organism	$Q_a$	$Q_b$	$Q_A$	$Q_B$	SI
0.40%	SA	200	1600	20	3980	2.59
"	ECOLI	100	800	20	3980	5.18
"	PSA	1200	1600	20	3980	2.50
"	PC	1800	1600	20	3980	2.50
"	CAN	100	16000	20	3980	0.45
"	AN	100	16000	20	3980	0.45

Use Level	Organism	$Q_a$	$Q_b$	$Q_A$	$Q_B$	SI
0.50%	SA	200	1600	25	4975	3.23
"	ECOLI	100	800	25	4975	6.47
"	PSA	1200	1600	25	4975	3.13
"	PC	1800	1600	25	4975	3.12
"	CAN	100	16000	25	4975	0.56
"	AN	100	16000	25	4975	0.56

TABLE 12  
100:1 Wt. Ratio DMDMH/IPBC

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.01%	SA	200	1600	1	99	0.07
"	ECOLI	100	800	1	99	0.13
"	PSA	1200	1600	1	99	0.06
"	PC	1800	1600	1	99	0.06
"	CAN	100	16000	1	99	0.02
"	AN	100	16000	1	99	0.02

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.025%	SA	200	1600	2.5	248	0.17
"	ECOLI	100	800	2.5	248	0.33
"	PSA	1200	1600	2.5	248	0.16
"	PC	1800	1600	2.5	248	0.16
"	CAN	100	16000	2.5	248	0.04
"	AN	100	16000	2.5	248	0.04

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.05%	SA	200	1600	5	495	0.33
"	ECOLI	100	800	5	495	0.67
"	PSA	1200	1600	5	495	0.31
"	PC	1800	1600	5	495	0.31
"	CAN	100	16000	5	495	0.08
"	AN	100	16000	5	495	0.08



TABLE 12 (CONT)

Use Level	Organism	$Q_a$	$Q_b$	$Q_A$	$Q_B$	SI
0.10%	SA	200	1600	10	990	0.67
"	ECOLI	100	800	10	990	1.34
"	PSA	1200	1600	10	990	0.63
"	PC	1800	1600	10	990	0.62
"	CAN	100	16000	10	990	0.16
"	AN	100	16000	10	990	0.16

Use Level	Organism	$Q_a$	$Q_b$	$Q_A$	$Q_B$	SI
0.20%	SA	200	1600	20	1980	1.34
"	ECOLI	100	800	20	1980	2.68
"	PSA	1200	1600	20	1980	1.25
"	PC	1800	1600	20	1980	1.25
"	CAN	100	16000	20	1980	0.32
"	AN	100	16000	20	1980	0.32

Use Level	Organism	$Q_a$	$Q_b$	$Q_A$	$Q_B$	SI
0.40%	SA	200	1600	40	3960	2.68
"	ECOLI	100	800	40	3960	5.35
"	PSA	1200	1600	40	3960	2.51
"	PC	1800	1600	40	3960	2.50
"	CAN	100	16000	40	3960	0.65
"	AN	100	16000	40	3960	0.65

Use Level	Organism	$Q_a$	$Q_b$	$Q_A$	$Q_B$	SI
0.50%	SA	200	1600	50	4950	3.34
"	ECOLI	100	800	50	4950	6.69
"	PSA	1200	1600	50	4950	3.14
"	PC	1800	1600	50	4950	3.12
"	CAN	100	16000	50	4950	0.81
"	AN	100	16000	50	4950	0.81

TABLE 13  
50:1 Wt. Ratio DMDMH/IPBC

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.01%	SA	200	1600	2	98	0.07
"	ECOLI	100	800	2	98	0.14
"	PSA	1200	1600	2	98	0.06
"	PC	1800	1600	2	98	0.06
"	CAN	100	16000	2	98	0.03
"	AN	100	16000	2	98	0.03

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.025%	SA	200	1600	5	245	0.18
"	ECOLI	100	800	5	245	0.36
"	PSA	1200	1600	5	245	0.16
"	PC	1800	1600	5	245	0.16
"	CAN	100	16000	5	245	0.07
"	AN	100	16000	5	245	0.07

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.05%	SA	200	1600	10	490	0.36
"	ECOLI	100	800	10	490	0.71
"	PSA	1200	1600	10	490	0.31
"	PC	1800	1600	10	490	0.31
"	CAN	100	16000	10	490	0.13
"	AN	100	16000	10	490	0.13

TABLE 13 (CONT)

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.10%	SA	200	1600	20	980	0.71
"	ECOLI	100	800	20	980	1.43
"	PSA	1200	1600	20	980	0.63
"	PC	1800	1600	20	980	0.62
"	CAN	100	16000	20	980	0.26
"	AN	100	16000	20	980	0.26

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.20%	SA	200	1600	40	1960	1.43
"	ECOLI	100	800	40	1960	2.85
"	PSA	1200	1600	40	1960	1.26
"	PC	1800	1600	40	1960	1.25
"	CAN	100	16000	40	1960	0.52
"	AN	100	16000	40	1960	0.52

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.40%	SA	200	1600	80	3920	2.85
"	ECOLI	100	800	80	3920	5.70
"	PSA	1200	1600	80	3920	2.52
"	PC	1800	1600	80	3920	2.49
"	CAN	100	16000	80	3920	1.05
"	AN	100	16000	80	3920	1.05

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.50%	SA	200	1600	100	4900	3.56
"	ECOLI	100	800	100	4900	7.13
"	PSA	1200	1600	100	4900	3.15
"	PC	1800	1600	100	4900	3.12
"	CAN	100	16000	100	4900	1.31
"	AN	100	16000	100	4900	1.31

TABLE 14  
20:1 Wt. Ratio DMDMH/IPBC

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.01%	SA	200	1600	5	95	0.08
"	ECOLI	100	800	5	95	0.17
"	PSA	1200	1600	5	95	0.06
"	PC	1800	1600	5	95	0.06
"	CAN	100	16000	5	95	0.06
"	AN	100	16000	5	95	0.06

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.025%	SA	200	1600	12.5	237.5	0.21
"	ECOLI	100	800	12.5	237.5	0.42
"	PSA	1200	1600	12.5	237.5	0.16
"	PC	1800	1600	12.5	237.5	0.16
"	CAN	100	16000	12.5	237.5	0.14
"	AN	100	16000	12.5	237.5	0.14

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.05%	SA	200	1600	25	475	0.42
"	ECOLI	100	800	25	475	0.84
"	PSA	1200	1600	25	475	0.32
"	PC	1800	1600	25	475	0.31
"	CAN	100	16000	25	475	0.28
"	AN	100	16000	25	475	0.28

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.10%	SA	200	1600	50	950	0.84
"	ECOLI	100	800	50	950	1.69
"	PSA	1200	1600	50	950	0.64
"	PC	1800	1600	50	950	0.62
"	CAN	100	16000	50	950	0.56
"	AN	100	16000	50	950	0.56

TABLE 14 (CONT)

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.20%	SA	200	1600	100	1900	1.69
"	ECOLI	100	800	100	1900	3.38
"	PSA	1200	1600	100	1900	1.27
"	PC	1800	1600	100	1900	1.24
"	CAN	100	16000	100	1900	1.12
"	AN	100	16000	100	1900	1.12

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.40%	SA	200	1600	200	4800	4.00
"	ECOLI	100	800	200	4800	8.00
"	PSA	1200	1600	200	4800	3.17
"	PC	1800	1600	200	4800	3.11
"	CAN	100	16000	200	4800	2.30
"	AN	100	16000	200	4800	2.30

<u>Use Level</u>	<u>Organism</u>	<u>Q<sub>a</sub></u>	<u>Q<sub>b</sub></u>	<u>Q<sub>A</sub></u>	<u>Q<sub>B</sub></u>	<u>SI</u>
0.50%	SA	200	1600	250	4750	4.22
"	ECOLI	100	800	250	4750	8.44
"	PSA	1200	1600	250	4750	3.18
"	PC	1800	1600	250	4750	3.11
"	CAN	100	16000	250	4750	2.80
"	AN	100	16000	250	4750	2.80

Similar SI results also were found with GADM and SUTTOCID® A as the methylol compound in place of Germall® II or DMDMH in admixtures with IPBC over the same wt. ratios and use level ranges as shown in the Tables 1-14 above.

Tables 1 through 14 above illustrate the synergism of IPBC (compound B) with Germall II or DMDMH (compound A) at weight ratios of A:B of 2000:1, 1000:1, 500:1, 200:1, 100:1, 50:1 and 20:1. Synergism is very effective for all ratios at low use levels, e.g. 0.01% to 0.1%, against all tested gram-positive, gram-negative organisms and fungi organisms. At slightly higher use concentrations, e.g. 0.20 to 0.50%, all tested ratios were synergistic against *Candida albicans* and *A. niger* also. However, at 50:1 and 20:1 ratios, the synergistic effect is negligible at the 0.01 to 0.1% use levels, and non-synergistic even against *Candida albicans* and *A. niger* at use levels of 0.20 to 0.50%.

The SI values were lower for Germall® II as the methylol compound in the admixtures as compared to DMDMH.

Similar results were obtained when iodopropynyl alcohol (IPGA) was substituted for IPBC in the admixtures described above.

### 3. PRESERVATIVE ACTIVITY (CHALLENGE TEST)

A typical cosmetic emulsion was prepared for microbiological challenge testing and predetermined admixtures of a methylol compound and IPBC were added at various use levels. The emulsion thus prepared had the following composition:

<u>Phase A</u>	<u>% wt.</u>
Stearic Acid	5.00
Mineral Oil	2.50
Cetyl Alcohol	1.00
Lareth-5 and Ceteth-5 and Oleth-5 and Steareth-5	0.50
Glycerol Monostearate and Polyoxyethylene Stearate	1.50

Phase B

Deionized Water	88.0
Triethanolamine 99%	1.00
Citric Acid 30% aqueous solution	0.60
Preservative Admixture	qs

To prepare the emulsion, Phases A and B were heated separately to 75-80°C. Phase A then was added to Phase B with mixing. The mixture then was cooled to 55-60°C. At this point the desired amount of the preservative admixture was added and the product was cooled to 50°C. while stirring. The citric acid solution then was added to adjust the pH and the mixture was stirred until a temperature of 30°C. was reached.

The challenge tests were carried out using the following microorganisms: SA, ECOLI, PSA, PC, AN and CAN, in this manner. 50 g. aliquots of the test emulsion containing various amounts of the preservative admixture were inoculated with approximately  $10^7$ - $10^8$  of the challenge organisms. The test samples then were stirred to disperse the challenge inoculum. The samples were incubated and assayed at 48 hours, 7, 14, 21 and 28 days. The assays were performed on 1 g. of the test sample by serially diluting  $10^1$  to  $10^6$  of the original concentration. The plating medium for bacteria was Lethen agar and for fungi it was low pH Mycophil agar with Tween 20. Each plated sample was incubated for 48 hours at 37°C. for bacteria, 5 days at 25°C. for mold, and 3 days at 25°C. for fungi. After incubation, readings of the number of colonies per milliliter (cfu/ml) were made. At 21 days the test product was reinoculated with half of the original inoculum. The data is presented in Tables 15-23 below.

TABLE 15  
2000:1 GERMALLO II/IPBC

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.01%	AN	69,000	260,000	190,000	17,000	4,500
"	CAN	98,000	76,000	1,400	3,100	19,000
"	ECOLI	110,000	290,000	2,400	138,000	560,000
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	190,000	220	<10	<10	3,700

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.025%	AN	2,800	10	10	<10	220
"	CAN	58,000	29,000	18,000	56,000	110,000
"	ECOLI	39,000	10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	37,000	170	<10	<10	<10

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.05%	AN	20	<10	<10	<10	<10
"	CAN	19,000	6,600	70	<10	320
"	ECOLI	3,400	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	31,000	<10	<10	<10	<10



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TABLE 15 (CONT)

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.1%	AN	<10	<10	<10	<10	<10
"	CAN	180	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	750	<10	<10	<10	<10

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.2%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10

Unpreserved control

<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
AN	52,000	27,000	19,000	19,000	19,000
CAN	110,000	130,000	240,000	180,000	240,000
ECOLI	54,000	140,000	170,000	170,000	74,000
PC	6,400,000	6,400,000	2,000,000	6,700,000	29,000
PSA	110,000	700	110,000	290,000	85,000
SA	2,800,000	250,000	51,000	3,700	330

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TABLE 15 (CONT)

<u>Inoculum Concentration</u>	
<u>Organism</u>	<u>0 Hours</u>
AN	26,000
CAN	1,000,000
ECOLI	3,600,000
PC	3,400,000
PSA	4,500,000
SA	4,100,000
	<u>21 Days</u>
	53,000
	1,900,000
	170,000
	87,000
	390,000
	200,000

TABLE 161000:1 GERMALLO II/IPBC

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.01%	AN	34,000	3,500	80	10	<10
"	CAN	420,000	24,000	950	6,400	6,400,000
"	ECOLI	120,000	63,000	93,000	92,000	9,600,000
"	PC	10	25,000	1,600	15,800	33,000,000
"	PSA	<10	<10	<10	<10	<10
"	SA	100,000	1,400	<10	<10	5,000

TABLE 16 (CONT)

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.025%	AN	530	10	<10	<10	<10
"	CAN	34,000	750	10	770	240,000
"	ECOLI	120,000	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	37,000	170	<10	<10	<10
<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.05%	AN	<10	<10	<10	<10	<10
"	CAN	13,000	<10	<10	<10	<10
"	ECOLI	68,000	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	21,000	<10	<10	<10	<10
<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.1%	AN	<10	<10	<10	<10	<10
"	CAN	10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	1,400	<10	<10	<10	<10

TABLE 16 (CONT.)

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.2%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10
<u>Unpreserved control</u>						
<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>	
AN	52,000	27,000	19,000	19,000	19,000	
CAN	110,000	130,000	240,000	180,000	240,000	
ECOLI	54,000	140,000	170,000	170,000	74,000	
PC	6,400,000	6,400,000	2,000,000	6,700,000	29,000	
PSA	110,000	700	110,000	290,000	85,000	
SA	2,800,000	250,000	51,000	3,700	330	
<u>Inoculum Concentration</u>						
<u>Organism</u>	<u>0 Hours</u>	<u>21 Days</u>				
AN	26,000	53,000				
CAN	1,000,000	1,900,000				
ECOLI	3,600,000	170,000				
PC	3,400,000	87,000				
PSA	4,500,000	390,000				
SA	4,100,000	200,000				

**TABLE 17**  
**500:1 GERMALLO II/IPBC**

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.01%	AN	23,000	40	<10	<10	<10
"	CAN	170,000	5,600	290	200	<10
"	ECOLI	90,000	57,000	95,000	70,000	240,000
"	PC	10	<10	<10	>10,000	42,000,000
"	PSA	<10	<10	<10	<10	<10
"	SA	380,000	440	<10	<10	5,100

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.05%	AN	<10	<10	<10	<10	<10
"	CAN	8,700	<10	<10	<10	<10
"	ECOLI	60,000	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	31,000	<10	<10	<10	<10

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.1%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	890	<10	<10	<10	<10

TABLE 17 (CONT.)

<u>Test L vel</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.2%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10
<u>Unpreserved control</u>						
<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>	
AN	6,100	520,000	18,000	5,000	11,000	
CAN	1,000,000	710,000	95,000	12,000	64,000	
ECOLI	7,100,000	6,200,000	610,000	350,000	120,000	
PC	14,600,000	160,000,000	3,600,000	2,720,000	9,500,000	
PSA	20	900	130	4,100	>100,000	
SA	43,000,000	600,000	1,000	220	<10	
<u>Inoculum Concentration</u>						
<u>Organism</u>	<u>0 Hours</u>	<u>21 Days</u>				
AN	19,000	4,700,000				
CAN	340,000	16,000,000				
ECOLI	3,900,000	1,480,000				
PC	3,800,000	1,380,000				
PSA	9,200,000	730,000				
SA	4,800,000	360,000				

**TABLE 18**  
**200:1 GERMALLO II/IPBC**

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.01%	AN	47,000	320	10	<10	<10
"	CAN	810,000	450,000	410,000	190,000	63,000
"	ECOLI	220,000	7,600	<10	850	>1,000,000
"	PC	10,000	500,000	1,900,000	1,100,000	193,000
"	PSA	<10	<10	<10	<10	<10
"	SA	190,000	23,000	120	<10	<10
<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.05%	AN	<10	<10	<10	<10	<10
"	CAN	190	<10	<10	<10	<10
"	ECOLI	37,000	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	19,000	<10	<10	<10	<10
<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.1%	AN	<10	<10	<10	<10	<10
"	CAN	10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	45,000	<10	<10	<10	<10

TABLE 18 (CONT.)

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.2%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10
<u>Unpreserved control</u>						
<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>	
AN	89,000	32,000	22,000	16,000	16,000	
CAN	210,000	670,000	430,000	590,000	640,000	
ECOLI	640,000	360,000	410,000	990,000	68,000	
PC	19,000,000	3,200,000	7,000,000	>10,000	2,760,000	
PSA	80	9,400	200,000	>10,000	34,000	
SA	6,300,000	190,000	11,000	580	120	
<u>Inoculum Concentration</u>						
<u>Organism</u>	<u>0 Hours</u>	<u>21 Days</u>				
AN	41,000	32,000				
CAN	640	1,100,000				
ECOLI	5,800,000	1,300,000				
PC	900,000	3,000,000				
PSA	1,800,000	4,900,000				
SA	7,200,000	2,000,000				



TABLE 19

## 50:1 GERMALLO II/IPBC

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.01%	AN	4,100	40	<10	<10	<10
"	CAN	310,000	7,700	560	5,600	5,200
"	ECOLI	170,000	710	10	<10	120
"	PC	7,400	74,000	340,000	720,000	520,000
"	PSA	<10	<10	<10	<10	30
"	SA	110,000	11,000	<10	<10	9,200
<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.05%	AN	<10	100	<10	<10	<10
"	CAN	210	<10	<10	<10	<10
"	ECOLI	150,000	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	35,000	<10	<10	<10	<10
<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.1%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	510	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	3,000	<10	<10	<10	<10

TABLE 19 (CONT)

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.2%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10
<u>Unpreserved control</u>						
<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>	
AN	89,000	32,000	22,000	16,000	16,000	
CAN	210,000	670,000	430,000	590,000	640,000	
ECOLI	640,000	360,000	410,000	990,000	68,000	
PC	19,000,000	3,200,000	7,000,000	>10,000	2,760,000	
PSA	80	9,400	200,000	>10,000	34,000	
SA	6,300,000	190,000	11,000	580	120	
<u>Inoculum Concentration</u>						
<u>Organism</u>	<u>0 Hours</u>	<u>21 Days</u>				
AN	41,000	32,000				
CAN	640	1,100,000				
ECOLI	5,800,000	1,300,000				
PC	900,000	3,000,000				
PSA	1,800,000	4,900,000				
SA	7,200,000	2,000,000				

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**TABLE 20**  
**20:1 GERMALLO II/IPBC**

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.01%	AN	3,100	<10	<10	<10	<10
"	CAN	75,000	220	<10	<10	2,400
"	ECOLI	160,000	110	<10	<10	20
"	PC	12,000	1,000,000	2,100,000	>1,000,000	730,000
"	PSA	<10	<10	<10	<10	4,000
"	SA	140,000	4,100	<10	<10	1,680

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.05%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	16,000	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	31,000	<10	<10	<10	<10

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.1%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	6,800	<10	<10	<10	<10

TABLE 20 (CONT.)

Test Level	Organism	48 Hours	7 Days	14 Days	21 Days	28 Days
0.2%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10
<u>Unpreserved control</u>						
Organism	48 Hours	7 Days	14 Days	21 Days	28 Days	
AN	89,000	32,000	22,000	16,000	16,000	
CAN	210,000	670,000	430,000	590,000	640,000	
ECOLI	640,000	360,000	410,000	990,000	68,000	
PC	19,000,000	3,200,000	7,000,000	>10,000	2,760,000	
PSA	80	9,400	200,000	>10,000	34,000	
SA	6,300,000	190,000	11,000	580	120	
<u>Inoculum Concentration</u>						
Organism	0 Hours	21 Days				
AN	41,000	32,000				
CAN	640	1,100,000				
ECOLI	5,800,000	1,300,000				
PC	900,000	3,000,000				
PSA	1,800,000	4,900,000				
SA	7,200,000	2,000,000				

**TABLE 21**  
**2000:1 DMDMH/IPBC**

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.025%	AN	4,100	38,000	270	80	100
"	CAN	270,000	1,900,000	550,000	220,000	210,000
"	ECOLI	1,300,000	<10	<10	<10	3,000
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	42,000	20	<10	<10	<10
<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.05%	AN	<10	<10	<10	<10	<10
"	CAN	770,000	130,000	670,000	160,000	64,000
"	ECOLI	220,000	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	9,000	<10	<10	<10	<10
<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.1%	AN	<10	<10	<10	<10	<10
"	CAN	580	840	500	40,000	83,000
"	ECOLI	340	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	29,000	<10	<10	<10	<10

TABLE 21 (CONT.)

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.2%	AN	<10	<10	<10	<10	<10
"	CAN	<10	10	10	<10	21
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10
<u>Unpreserved control</u>						
<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>	
AN	37,000	36,000	24,000	5,200	6,000	
CAN	120,000	1,900,000	3,300,000	480,000	790,000	
ECOLI	150,000	2,500,000	7,300,000	240,000	140,000	
PC	19,000,000	15,600,000	5,900,000	8,500,000	31,000,000	
PSA	<10	<10	100	15,200	300,000	
SA	7,000,000	>1,000,000	12,000	3,000	110	
<u>Inoculum Concentration</u>						
<u>Organism</u>	<u>0 Hours</u>	<u>21 Days</u>				
AN	50,000	41,000				
CAN	1,400,000	640				
ECOLI	4,800,000	5,800,000				
PC	9,200,000	900,000				
PSA	6,900,000	9,000,000				
SA	5,700,000	7,200,000				

**TABLE 22**  
**1000:1 DMDMH/IPBC**

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.025%	AN	650	900	<10	<10	140
"	CAN	97,000	900,000	2,300,000	120,000	120,000
"	ECOLI	160,000	<10	<10	<10	2,400
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	23,000	<10	<10	<10	90

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.05%	AN	20	<10	<10	<10	<10
"	CAN	65,000	520,000	56,000	32,000	370,000
"	ECOLI	26,000	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	12,000	<10	<10	<10	<10

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.1%	AN	<10	<10	<10	<10	<10
"	CAN	3,100	4,800	180	640	4,400
"	ECOLI	45,000	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	6,000	<10	<10	<10	<10

TABLE 22 (CONT.)

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.2%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	180	<10	<10	<10	<10
<u>Unpreserved control</u>						
<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>	
AN	37,000	36,000	24,000	5,200	6,000	
CAN	120,000	1,900,000	3,300,000	480,000	790,000	
ECOLI	150,000	2,500,000	7,300,000	240,000	140,000	
PC	19,000,000	15,600,000	5,900,000	8,500,000	31,000,000	
PSA	<10	<10	100	15,200	300,000	
SA	7,000,000	>1,000,000	12,000	3,000	110	
<u>Inoculum Concentration</u>						
<u>Organism</u>	<u>0 Hours</u>	<u>21 Days</u>				
AN	50,000	41,000				
CAN	1,400,000	640				
ECOLI	4,800,000	5,800,000				
PC	9,200,000	900,000				
PSA	6,900,000	9,000,000				
SA	5,700,000	7,200,000				



TABLE 23  
2000:1 GADM/IPBC

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.025%	AN	300	21	10	<10	11
"	CAN	480,000	890,000	940,000	1,040,000	130,000
"	ECOLI	230,000	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	78,000	<10	<10	<10	<10

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.05%	AN	<10	<10	<10	<10	<10
"	CAN	110,000	4,100,000	3,600,000	330,000	97,000
"	ECOLI	120,000	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	56,000	<10	<10	<10	<10

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.1%	AN	<10	<10	<10	<10	<10
"	CAN	1,200,000	53,000	430,000	144,000	110,000
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10

TABLE 23 (CONT)

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.2%	AN	<10	<10	<10	<10	<10
"	CAN	90,000	<10	<10	<10	60
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10
<u>Unpreserved control</u>						
<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>	
AN	50,000	33,000	33,000	13,000	5,400	
CAN	780,000	780,000	780,000	500,000	170,000	
ECOLI	600,000	3,100,000	920,000	920,000	140,000	
PC	11,000,000	30,000,000	10,000,000	10,000,000	1,400,000	
PSA	3,800	600	12,800	12,800	100,000	
SA	14,000,000	410,000	7,100	7,100	80	
<u>Inoculum Concentration</u>						
<u>Organism</u>	<u>0 Hours</u>	<u>21 Days</u>				
AN	53,000	10,000				
CAN	1,900,000	310,000				
ECOLI	170,000	3,500,000				
PC	87,000	2,500,000				
PSA	390,000	5,400,000				
SA	200,000	4,100,000				

### Discussion of Challenge Testing Results

The 28-day challenge results reported in Tables 15-23 above demonstrate the effectiveness of the preservative admixture of the invention in a use emulsion composition against a wide range of bacteria and fungi organisms.

For example, admixture compositions of Germall® II and IPBC at a wt. ratio of 2000:1 (Table 15), when present at use levels of 0.05 to 0.2%, corresponding to 0.75 to 10 ppm IPBC and 500 to 2000 ppm methylol levels, provide substantially complete protection against all tested organisms after 28 days. At the low use level of 0.05% active, all the challenge tests passed within 21 days. Then, upon reinoculation after 21 days, all organisms died within 7 days except CAN which cleared within 14 days.

Table 21 shows the challenge test results for DMDMH and IPBC admixtures at the same 2000:1 wt. ratio. A use level of 0.2%, however, is needed for this blend to pass against all organisms after 21 days. Upon reinoculation, all organisms died within 7 days with the exception of CAN which cleared within 14 days. It is thus evident from these results that Germall® II blended with IPBC is 4 times more effective than a DMDMH/IPBC blend.

Germall® II also is superior to GADM as the methylol compound, as shown in Table 23.

Table 24 below is a study of the activity of solution of GII/IPBC in propylene glycol. The admixtures of the active GII and IPBC components were prepared at weight ratios of 99.5%/0.5% and 99%/1%, and added to 60% by weight propylene glycol. The resulting solutions were tested at 0.05%, 0.1% and 0.2% total active in a proteinaceous shampoo formulation.

TABLE 24

99.5% GII/0.5% IPBC/propylene glycol solution - 0.05% total active

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.05%	AN	310,000	2,000	<10	<10	<10
"	CAN	44,000	2,400	<10	<10	1,800
"	ECOLI	3,000	<10	<10	<10	<10
"	PC	400,000	62,000	90	<10	>10,000
"	PSA	5,300,000	3,000	<10	<10	>10,000
"	SA	10	10	<10	<10	<10

99.5% GII/0.5% IPBC/propylene glycol solution - 0.1% total active

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.1%	AN	210,000	<10	<10	<10	<10
"	CAN	1,500	<10	<10	<10	<10
"	ECOLI	580	<10	<10	<10	20
"	PC	34,000	<10	<10	<10	<10
"	PSA	780	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10

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## 99.5% GII/0.5% IPBC/propylene glycol solution - 0.2% total active

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.2%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10

## 99% GII/1% IPBC/propylene glycol solution - 0.05% total active

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.05%	AN	89,000	<10	<10	<10	<10
"	CAN	4,400	<10	<10	<10	20
"	ECOLI	3,300	<10	<10	<10	60
"	PC	260,000	<10	<10	<10	3,400
"	PSA	64,000	<10	<10	<10	280
"	SA	<10	<10	<10	<10	<10

## 99% GII/1% IPBC/propylene glycol solution - 0.1% total active

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.1%	AN	37,000	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	840	<10	<10	<10	<10
"	PC	48,000	<10	<10	<10	<10
"	PSA	440	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10

99% GII/1% IPBC/propylene glycol solution - 0.2% total active

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.2%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10

Unpreserved control

<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
AN	4,000,000				
CAN	160,000	>100,000,000	5,600,000	>100,000,000	>1,000,000
ECOLI		>100,000,000	800,000,000	48,000,000	600,000
PC	>100,000,000	50,000,000	48,000,000		>1,000,000
PSA	>100,000,000	>100,000,000	58,000,000	65,000,000	>1,000,000
SA		>100,000,000			

Inoculum Concentration

<u>Organism</u>	<u>0 Hours</u>	<u>21 Days</u>
AN	380,000	330,000
CAN	860,000	4,200,000
ECOLI	2,500,000	5,300,000
PC	1,900,000	60,000,000
PSA	3,200,000	5,000,000
SA	2,400,000	3,000,000

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Table 25 shows a similar study as in Table 24 above in which the vehicle for the composition was the typical emulsion described above.

TABLE 2599.5% GII/0.5% IPBC/propylene glycol solution - 0.05% total active

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.05%	AN	40	<10	<10	<10	<10
"	CAN	520,000	<10	<10	<10	<10
"	ECOLI	320	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	24,000	<10	<10	<10	<10

99.5% GII/0.5% IPBC/propylene glycol solution - 0.1% total active

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.1%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	650	<10	<10	<10	<10



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99.5% GII/0.5% IPBC/propylene glycol solution - 0.2% total active

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.2%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10

99% GII/1% IPBC/propylene glycol solution - 0.05% total active

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.05%	AN	<10	<10	<10	<10	<10
"	CAN	6,700	<10	<10	<10	<10
"	ECOLI	66,000	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	50,000	<10	<10	<10	<10

99% GII/1% IPBC/propylene glycol solution - 0.1% total active

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.1%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	620	<10	<10	<10	<10

99% GII/1% IPBC/propylene glycol solution - 0.2% total active

<u>Test Level</u>	<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
0.2%	AN	<10	<10	<10	<10	<10
"	CAN	<10	<10	<10	<10	<10
"	ECOLI	<10	<10	<10	<10	<10
"	PC	<10	<10	<10	<10	<10
"	PSA	<10	<10	<10	<10	<10
"	SA	<10	<10	<10	<10	<10

Unpreserved control

<u>Organism</u>	<u>48 Hours</u>	<u>7 Days</u>	<u>14 Days</u>	<u>21 Days</u>	<u>28 Days</u>
AN	3,100,000	650,000	370,000	1,400,000	260,000
CAN	6,000,000	4,000,000	1,100,000	5,800,000	>1,000,000
ECOLI	11,000,000	7,300,000	6,000,000	730,000	220,000
PC	100,000,000	53,000,000	40,000,000	40,000,000	600,000
PSA	5,000,000	500,000	2,700,000	72,000	20,000
SA	30,000,000	150,000	440,000	2,500	9,900

Inoculum Concentration

<u>Organism</u>	<u>0 Hours</u>	<u>21 Days</u>
AN	380,000	330,000
CAN	860,000	4,200,000
ECOLI	2,500,000	5,300,000
PC	1,900,000	60,000,000
PSA	3,200,000	5,000,000
SA	2,400,000	3,000,000

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The results shown in Tables 24 and 25 demonstrate that the compositions of the invention are completely effective against the tested organisms in comparison to the unpreserved controls.

While the invention has been described with particular reference to certain embodiments thereof, it will be understood that changes and modifications may be made which are within the skill of the art. Accordingly, it is intended to be bound only by the following claims, in which:

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## WHAT IS CLAIMED IS:

1. A water soluble preservative antimicrobial composition for addition to commercial use products at predetermined use levels to provide synergistic biocidal activity against a wide range of fungi and gram-negative and gram-positive bacteria, comprising

(1) an admixture of

(a) one or more methylol compounds, and

(b) 3-iodo-2-propynylbutyl carbamate,

in a weight ratio of (a):(b) of 100:1 to 2000:1.

2. A water soluble preservative admixture according to claim 1 wherein said weight ratio is 200:1 to 500:1.

3. A water soluble preservative admixture according to claim 1 wherein

(a) is N-[1,3-bis(hydroxymethyl)-2,5-dioxo-4-imidazolidinyl]-N,N'-bis(hydroxymethyl) urea, imidurea, 1,3-dimethylol-5,5-dimethyl hydantoin, sodium hydroxymethylglycinate, or glycine anhydride dimethylol.

4. A preservative admixture according to claim 3 wherein

(a) is N-[1,3-bis(hydroxymethyl)-2,5-dioxo-4-imidazolidinyl]-N,N'-bis(hydroxymethyl) urea.

5. A composition according to claim 1 which also includes (2) propylene glycol or 1,3-butylene glycol.

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6. A water soluble preservative antimicrobial composition according to claim 5 which comprises about 20 to 55 weight percent of (1) and 45 to 80 weight percent of (2).

7. A composition according to claim 6 which comprises about 40 weight percent of (1) and about 60 weight percent of (2).

8. A commercial use product which is protected for an extended period of time against contamination by a wide range of fungi and gram-negative and gram-positive bacteria which includes 0.01 to 0.5% by weight of the water soluble preservative composition of claims 1-7.

9. A commercial use product according to claims 1-8 which includes about 0.1% by weight of the water soluble preservative composition of claim 2 or 5.

10. A commercial use product according to claims 1-9 in which said composition is water solubilized and uniformly distributed throughout said composition.

11. A commercial use product according to claims 1-10 in which (b) is present therein in an amount of 0.5 to 10 ppm, and (a) is present in an amount of at least 250 ppm.

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12. A commercial use product according to claims 1-11 which is a personal care, household or industrial composition.

13. A commercial use product which is protected for an extended period of time against contamination by a wide range of fungi and gram-negative and gram-positive bacteria which includes 0.1 to 5% by weight of the composition of claims 1-12.

14. A product according to claim 13 which includes about 0.5 to 1% by weight of the composition of claim 1 or 5.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/04895

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : 514/252, 389, 390, 478, 479, 561

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/252, 389, 390, 478, 479, 561

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,844,891 (Rosen et. al.) 04 July 1989, see entire document.	1-14
Y	US, A, 3,987,184 (Foelsch) 19 October 1976, see entire document.	1-3 and 5-14
Y	US, A, 5,244,653 (Berke et. al.) 14 September 1993, see entire document.	1-3 and 5-14
Y	US, A, 4,337,269 (Berke et. al.) 29 June 1982, see entire document.	1-3 and 5-14
Y	US, A, 4,655,815 (Jakubowski) 07 April 1987, see entire document.	1-3 and 5-14



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

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Date of mailing of the international search report

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/04895

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A01N 37/12, 37/44, 43/50, 43/58, 43/60, 47/10; A61K 31/27, 31/50, 31/195, 31/415, 31/495